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GENETIC ASPECTS OF LARVAL GROWTH UNDER REDUCED SALINITY IN *MYTILUS EDULIS*

DAVID J. INNES[1] AND LESLIE E. HALEY

Department of Biology, Dalhousie University, Halifax, Nova Scotia

Although the ecology and physiology of adult *Mytilus edulis* have gained considerable attention over the years (reviewed in Bayne, 1976), much less information is available for the pelagic larval stage. Compared to some other invertebrate larvae, *Mytilus* larvae spend a relatively long period (three to several weeks) in the plankton. The ability of larvae to grow, successfully settle and develop into adults depends on their response to the range of temperature, salinity and food conditions during this planktonic stage. Fortunately, work by Bayne (1964, 1965, 1970) has established techniques for the routine culture of mussel larvae and provides basic information on the effects of food, temperature and salinity on larval growth. Brenko and Calabrese (1969) give some data on the combined effects of temperature and salinity on larval growth. Similar information is available for a number of bivalve species (Davis and Calabrese, 1964; Calabrese, 1969; Lough and Gonor, 1973).

Measuring larval response under different conditions determines the effect environmental parameters can have on observed variation in quantitative traits, such as growth. A factor not taken into account in studies on marine invertebrate larvae is the effect genetic variation and its interaction with environmental conditions have on larval growth. This is usually ignored or at best treated as within-population variation by using a pool of larvae derived from several sets of parents. Partitioning observed variation into its components is necessary for a more complete understanding of the role genetic variation plays in determining phenotypic variation in various traits of individuals in a population. The relationship between phenotypic variation and genetic variation is fundamental to studying the adaptation and evolution of organisms with reference to particular environmental situations (Lewontin, 1974).

A genetic component of variation and its interaction with the salinity environment can be estimated for larval growth in a population of *Mytilus*. This involves raising individual families of larvae of known parentage under different salinity conditions. Breeding techniques have now been used in several species as a means of investigating quantitative variation in marine organisms (Chapman, 1974; Doyle, 1974; McLaren, 1976; Newkirk, Haley, Waugh and Doyle, 1977). *Mytilus edulis* is particularly suitable, not only because of its well-studied biology, but because individuals release large amounts of gametes, allowing for multiple matings and more efficient experimental designs.

[1] Present address: Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, New York 11794

Materials and Methods

Field collections

Adult specimens of *M. edulis* were collected from Ostrea Lake, a shallow, enclosed bay with a narrow opening near the mouth of Musquodoboit Harbour, Nova Scotia. An idea of the salinity and temperature characteristics of this habitat is given in Figure 1 (data provided by L. MacLeod, Nova Scotia Department of Fisheries, Halifax, personal communication) Natural spawning usually begins here in early June, when the water temperature reaches 14–15° C (MacLeod, personal communication). Mussels described in these experiments were collected a few months prior to natural spawning and maintained in running sea water in the laboratory. Gamete maturation was accelerated by increasing the water temperature in steps over a few weeks from 4° C to about 12° C. During this period the mussels were fed regularly a suspension of algae (mostly *Tetraselmis* sp.). Spawnable individuals were obtained well before those in the natural population.

Raising the larvae

Larvae were cultured using the techniques of Bayne (1965). Spawning was induced by placing individuals in heated (23° C), filtered sea water. Usually spawning began within half an hour. Eggs from each female were pipetted and suspended into individual beakers of filtered sea water (from a 5 μm filter). These were then divided equally among several 1000 ml beakers (depending on the experimental design). The eggs were fertilized by adding about 1 ml of sperm suspension and evenly mixing it with the eggs. Beakers of fertilized eggs were held at 16° C for the first 48 hours of development.

Density of the swimming veliger larvae was estimated 48 hours after fertilization and new beakers were set up at about 20 larvae/ml in 900 ml of sea water. The larvae were fed a suspension of a single-celled alga, *Isochrysis galbana*, at a concentration of about 80 cells/μl in each beaker. Every 48 hours the water in each beaker was passed through a 44 μm nitex screen to retain the larvae. Each beaker was rinsed with distilled water followed by sea water. The larvae were then resuspended in filtered sea water with sufficient *Isochrysis* to give the desired cell concentration. Antibiotics were used, with Penicillin-G and Streptomysin sulfate at concentrations of 50.8 mg/liter and 22.2 mg/liter, respectively. Between such changes, the beakers of larvae were maintained in a 16° C temperature-controlled room in the dark. Complete randomization was used at all stages of the experimental procedure. Larval growth was estimated by sampling larvae from each beaker at various times after fertilization and measuring the longest axis of 30 or 40 individuals on the projection screen of an inverted microscope.

Experiments

Two salinity experiments were conducted to investigate the effect of low salinity on larval growth. The first experiment involved three males crossed with a single female. The larvae were pooled 48 hours after fertilization to give a homogenous mixture of the three families. Four beakers of this pooled group were

set up at a salinity of 30‰, while two beakers were reduced to 18‰ by adding distilled water (salinities in all experiments were controlled within less than a part per thousand). Larval length was measured at 6, 12, and 18 days after fertilization.

In a second salinity experiment, a pooled group of larvae was produced from two males crossed to a single female. Three salinity treatments were used (30‰, 16‰, and 11‰) with two replicate beakers within each salinity. The two low salinity groups were first reduced to 20‰ three days after fertilization and on the fourth day reduced to the treatment levels of 16 and 11‰. An initial mean larval length was determined three days after fertilization for the pooled group. Subsequent measurements were made 6, 11, 15, and 19 days after fertilization.

Pooling families of larvae is an efficient way of estimating the response of a population to variations in salinity, but this procedure confounds genetic information with the unexplained within-treatment variation. Two experiments were designed to determine the genetic contribution to variation in larval growth and its interaction with salinity. The first experiment was a factorial mating in which six males and six females were crossed in all possible combinations. Each of the resulting 36 families were raised at two salinities (30‰ and 12‰) for 16 days, when mean larval length was measured. Eight males were paired at random with eight females in a second genetic experiment. These eight unrelated, single-pair families were raised under three salinity conditions (30‰, 18‰, and 12‰). After 16 days of growth, mean length was estimated from a sample of larvae from each beaker.

The components of variation in each genetic experiment were estimated by factorial analyses of variance (Sokal and Rholf, 1969). The first experiment was designed as a three-way, mixed model ANOVA with males and females as random

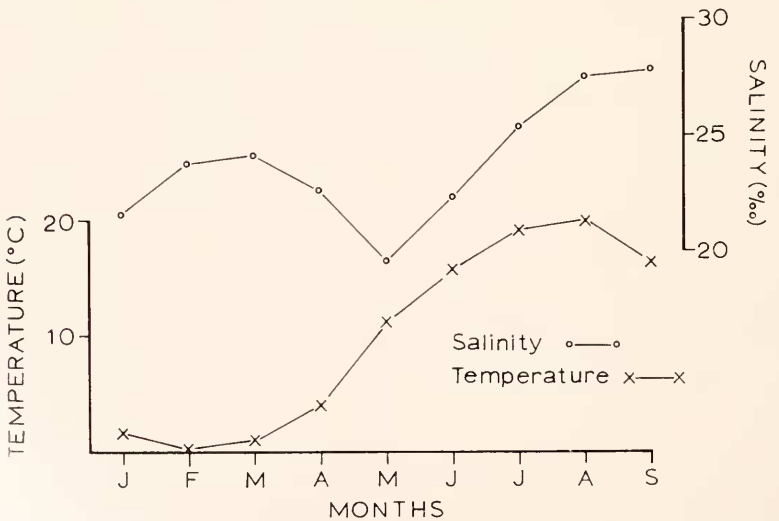

FIGURE 1. Monthly mean temperature and salinity for 1975 taken from a depth of 3 meters in Ostrea Lake, Nova Scotia. Standard errors for each month are all less than 3° C and 1‰.

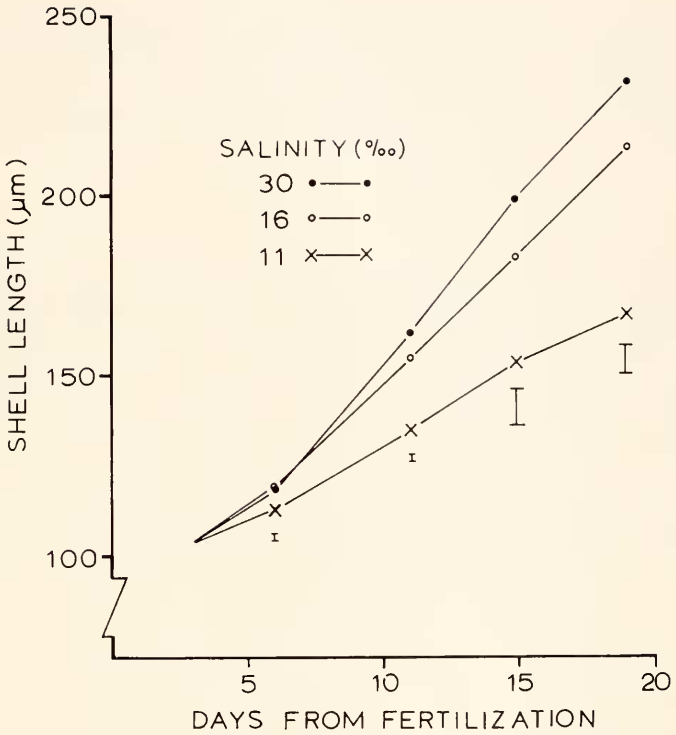

FIGURE 2. Increase in mean length of larvae raised at three salinities after development until day 3 at 30‰. Vertical bars indicate the least significant difference between means for each day ($\alpha = 0.05$).

effects and salinity as a fixed effect. In the single-pair experiment, random families and fixed salinities were used as main effects in a two-way mixed model design.

RESULTS

The effect of salinity

In the first salinity experiment no significant difference in mean larval length after 18 days of growth could be detected between larvae raised at 30‰ and those raised at 18‰. In the second experiment salinities lower than 18‰ produced a marked decrease in larval growth (Fig. 2). The first detectable response was noted three days after the salinity was dropped from 30‰ to 11‰. By day 11 a slower rate of growth was also detectable in the 16‰ treatment. Except for this slower growth, larvae in the two low salinity treatments showed no abnormal development, and swimming behavior was just as vigorous as in the 30‰ group. Although no counts were made, mortality appeared to be very low with no obvious difference among the three treatments.

These results differ from those of Brenko and Calabrese (1969), who found reduced growth at a salinity of 25‰. This may reflect genetic differences in larval

response to salinity between mussel populations. Unpublished observations and the work of Bayne (1965) suggest that larval growth at different salinities can depend on the population from which the parents were derived.

No significant difference was found among replicated beakers in these experiments allowing for a single beaker to be used for each treatment in the genetic experiments.

The genetic experiments

From the factorial mating experiment at two salinities males, females, salinities and all interactions had a significant effect on 16 day larval length (Table I). A substantial proportion (23%) of this larval variation could be accounted for by genetic and interaction differences among families. This is actually an underestimate, since some of this variation remains inseparable from the 77% within-family variation. Examining the components of variation showed males contributed about twice as much to the total as females (5.4% compared to 2.4%). This may be a function of the small number of males and females used rather than any differences due to sex alone. The interaction between males and females was slightly larger, making up 6.3% of the total variation. Families responded differently to changes in salinity indicating the presence of genetic interactions with salinity.

FIGURE 3. Mean length (1 unit = 2.74 μm) of 16 day old larvae from eight single-pair families raised at three salinities showing responses of families to salinities and interactions between families and salinities. Vertical bar indicates the least significant difference between means ($\alpha = 0.05$).

TABLE I

ANOVA *on 16 day larval length for factorial mating experiment raised at 30‰ and 12‰.*

Source of variation	df	Mean square	Component of variation (%)
Main effects			
Salinity (S)	1	99,322.8**	
Female (F)	5	268.5*	2.4
Male (M)	5	499.7**	5.4
2-way interactions			
S × F	5	165.0**	3.5
S × M	5	125.9**	2.5
F × M	25	91.9**	6.3
3-way interaction			
S × F × M	25	32.1**	3.2
Residual	2808	12.1	77.0
Total	2879		100.3

* $P < 0.05$.
** $P < 0.01$.

The salinity-female and salinity-male interactions were similar (3.5% and 2.5%, respectively) and seemed to be as important as the male and female components themselves. An appreciable (3.2%) component due to a salinity-male-female interaction was present.

The male and female effects and the male-female interaction effect are due to a genetic component of variation contributed by each individual parent in the experiment. Male and female variance components estimate 1/4 of the additive genetic variation, as well as any sex differences, while the male-female interaction estimates 1/4 of the dominance variation. The relative importance of additive genetic variance for a trait compared to the total phenotypic variation is a measure of its heritability in the population. Estimated heritabilities are used by quantitative geneticists to predict responses to selection. Such a detailed genetic analysis is beyond the scope of the present study, and a treatment of the subject can be found in Falconer (1960) and Becker (1975).

In the experiment involving eight single-pair families at three salinities, families, salinities and their interaction were all highly significant in influencing 16 day larval length (Table II). Variation among families represents a large portion of the genetic variation (1/2 additive and 1/4 dominance) and made up a substantial amount (27%) of the total observed variation in larval length. The interaction of this genetic component with salinity, although significant, was not as large (3% of the total). The remaining 70% could be attributed to variation within each family, comparable to the 77% of the previous experiment. Figure 3 plots family means at the three salinities, illustrating the sources of variation and their effect on larval length. The response to these salinities, averaged over families, showed no significant difference between the 18 and 30‰ salinity levels but a significant decrease

TABLE II

ANOVA *on 16 day larval length for single-pair families raised at 30‰, 18‰ and 12‰.*

Source of variation	df	Mean square	Component of variation (%)
Salinities (S)	2	14,478.1*	
Families (F)	7	1,288.1*	27
S × F	14	77.1*	3
Residual	936	27.8	70
Total	959		100

* $P < 0.01$.

in mean length at 12‰. This pattern agrees with the results found in the salinity experiments.

DISCUSSION

The salinity environment experienced by the Ostrea Lake mussel population during spawning is characterized by a low in the early spring, followed by a rise over the summer months (Fig. 1), a pattern that seems to be repeated each year (MacLeod, personal communication). Depending on when some adults spawned, larvae from this population may very well encounter salinities ranging from 18‰ to almost 30‰. Results of the experiments showed no average effect of these salinities on larval growth, suggesting that the population is prepared to cope with such a salinity range during the pelagic larval period. Salinities lower than this significantly decreased growth. A similar pattern of response was observed by Davis (1958) for larvae of *Venus mercenaria* and *Crassostrea virginica*.

There tends to be a general reduction in larval growth for bivalve species raised at reduced salinity (Davis, 1958; Davis and Ansell, 1962; Davis and Calabrese, 1964; Brenko and Calabrese, 1969; Calabrese, 1969; Lough and Gonor, 1971, 1973). In some cases this is accompanied by a large increase in mortality (Brenko and Calabrese, 1969; Calabrese, 1969), indicating that some sort of physiological stress may be hampering normal growth. In the present experiments with *Mytilus*, normal behavior was observed with no obvious mortality, even at the lowest salinity tested. Slow growth in this case could just be a nonfatal consequence of some physiological response mussel larvae have for surviving at reduced salinities. Little is known of larval physiology, making it difficult to speculate on a possible mechanism, although it may be related to energy requirements. Lough and Gonor (1973) measured oxygen consumption of *Adula californiensis* larvae exposed to different salinities, but could not detect any significant effect. This could possibly be due to poor culture conditions, since they observed very little growth over a sufficiently long period of time.

In a random sample of mussel larvae from a population, observed variation in a metric trait, such as larval length, is the result of genetic differences among individuals and differences in the microenvironment in which they have been growing. The two genetic experiments show that a substantial amount of this phenotypic variation is of genetic origin. This is consistent with the general con-

clusion from electrophoretic data that *Mytilus edulis* populations possess a large amount of genetic variability (Levinton and Koehn, 1976). Indeed, breeding studies themselves can be used as evidence for the existence of high levels of genetic variability in natural populations (Lewontin, 1974).

Families of larvae were raised at different salinities to determine if genetic response depends on salinity level. Significant interactions with salinity found in these experiments indicated the presence of genes, affecting larval growth, which depend on the level of the salinity environment for their expression (Falconer and Latyszewski, 1952; Scheinberg, 1973). That is, the relative importance of genes having an influence was different at the various salinities. This close connection between genes and salinity is not surprising, since salinity variations are very much a part of the estuarine habitat of *Mytilus*. In this fluctuating environment, the selection of genes which are sensitive to changes in salinity might be part of a mechanism for adjusting to different salinities (Speiss, 1968; Fontdevila, 1970). Laboratory studies on a number of organisms and traits have detected similar kinds of genotype-environment interaction (Speiss, 1968). Selection experiments on *Drosophila* at various temperatures indicated the presence of genes influencing wing length only at specific temperatures, as well as genes affecting wing length at all temperatures (Druger, 1962). The role of this additional source of genetic variation in populations and its relationship to changing environments is not clearly understood. Progress is being made using genetically defined laboratory populations, but little has been attempted with natural populations (Westerman and Lawrence, 1970; Westerman, 1971; Fontdevila, 1973).

Due to the small number of individuals represented in the experiments, it is difficult to attach any meaningful estimate to the Ostrea Lake population of the amount and kinds of genetic variability present. However, it must be reasonably high, as indicated by the highly significant results obtained. The importance of this genetic variability for larval growth appears to be almost equalled by its interaction with salinity. As pointed out by Westerman and Lawrence (1970), this interaction complicates a genetic analysis of the structure of a population under a single set of conditions. Its presence, however, brings up the question of its evolutionary significance. In the present study such interaction may in some way be related to past selective influences of the salinity environment on previous generations. Levins (1968) proposed that populations could be composed of a range of genotypes, each adapted to a specific environment through genotype-environment interaction, as an adaptation to a fluctuating environment. Without more extensive experiments, it is difficult to see an adaptive relationship between larval growth rate and salinity. Slower growth under certain salinity conditions, however, would extend the pelagic period (Bayne, 1965), increasing the probability of encountering a more "suitable" salinity environment for settlement. Salinity is, of course, only one of several important factors of concern to *Mytilus* larvae and adults (Scheltema, 1965).

With the increasing number of marine invertebrates which can be cultured through their complete life cycle, breeding studies are beginning to be used to obtain information on the genetic structure of various populations. This enables a valuable approach to understanding the relationship between genetic variability for specific traits of a species and the interaction with environmental parameters

thought to be of some importance. It would be interesting to extend the results obtained here to other populations of *Mytilus edulis* experiencing contrasting salinity conditions.

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Summary

1. Adult specimens of *Mytilus edulis* in spawning condition were collected from a shallow, enclosed bay which was known to experience fluctuations in salinity. Growth of the resulting larvae was measured under different salinity conditions in the laboratory.

2. There was no significant difference in mean length between larvae raised at 30‰ and those raised at 18‰ after 18 days of growth. Salinities below this significantly decreased growth.

3. Families of larvae of known parentage were raised at different salinities.

4. The genetic analysis indicated substantial genetic variation in larval length measured 16 days after fertilization, as well as significant genetic interaction with salinity.

5. This is interpreted as the presence of genes influencing larval growth which depend on salinity for their expression and may be related to the past selective influence of a fluctuating environment.

6. These quantitative genetic techniques provide a useful approach to studying genetic variation in marine organisms and its interaction with the environment.

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